

AMENDMENT

Kindly amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows:

IN THE CLAIMS:

Kindly amend the claims, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows:

1-40. (Cancelled)

41. (Currently amended) A method for the transformation of plastid genomes, comprising the steps of:

- a) providing a transformation vector carrying a DNA sequence of interest and a selection marker;
- b) subjecting a plant material derived from *Asteraceae*, which comprises plastids, to a transformation treatment in order to allow the plastids to receive the transformation vector;
- c) placing the thus treated plant material for a period of time into contact with a liquid culture medium without a selection agent;
- d) subsequently placing the plant material into contact with a liquid adding a selection agent to the culture medium comprising ~~a selection agent~~ the plant material; and
- e) refreshing the liquid culture medium comprising a selection agent to allow plant material comprising plastids that have acquired the DNA of interest to grow into transformants.

42. (Cancelled)

43. (Currently amended) The method as claimed in claim 41, wherein ~~the~~ an expression vector comprises:

an expression cassette which comprises optionally a promoter active in the plant species to be transformed, a DNA insertion site for receiving ~~the~~ a transforming DNA of interest, optionally one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a DNA sequence encoding a transcription termination region active in the plant species to be transformed,

optionally a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with ~~the~~ a plastid genome of interest, and

optionally a DNA sequence encoding a gene of interest inserted into the insertion site of the expression cassette.

44. (Previously presented) The method as claimed in claim 43, wherein the vector comprises the promoter, the DNA sequence encoding the gene of interest, the one or more selection markers and the set of DNA targeting segments.

45. (Currently amended) The method as claimed in claim 41, wherein the transformants carry the DNA of interest in ~~their~~ the genome of the transformant.

46. (Previously presented) The method as claimed in claim 41, wherein the plastids to be transformed are selected from the group consisting of chloroplasts, amyloplasts, elaioplasts, etioplasts, chromoplasts, leucoplasts and proplastids.

47. (Currently amended) The method as claimed in claim ~~41~~ 43, wherein ~~the~~ a promoter is selected from the group consisting of the chloroplast specific ribosomal RNA operon promoter *rrn*(16S rRNA), *psbA*, *rbcL*, *trnV* and *rps16*.

48. (Currently amended) The method as claimed in claim 41, wherein ~~the~~ a DNA of interest is a gene encoding a therapeutic or prophylactic (bio)pharmaceutical (poly)peptide.

49. (Currently amended) The method as claimed in claim 41, wherein ~~the~~ a DNA of interest is selected from the group consisting of genes encoding herbicide resistance, insect resistance, fungal resistance, bacterial resistance; genes that lead to stress tolerance, ~~for instance to cold, high salt or minerals~~; and genes that improve yield, starch accumulation, fatty acid accumulation or photosynthesis.

50. (Currently amended) The method as claimed in claim ~~41~~ 43, wherein the terminator is from a gene selected from the group consisting of the *psb A*, *rrn*, *rbcL*, *trnV* and *rps16*.

51. (Previously presented) The method as claimed in claim 41, wherein the selection marker is a gene conferring resistance against agents selected from the group consisting of spectinomycin, streptomycin, kanamycin, hygromycin and chloramphenicol, glyphosate and bialaphose.

52. (Previously presented) The method as claimed in claim 41, wherein the selection marker is a visual marker.

53. (Previously presented) A method for the transformation of plastid genomes of a plant species, comprising the steps of:

- a) providing a transformation vector carrying a DNA sequence of interest, and one or more selection markers;
- b) subjecting a plant material derived from *Asteraceae*, which comprises plastids, to a transformation treatment in order to allow the plastids to receive the transformation vector;
- c) placing the thus treated plant material for a period of time into contact with a culture medium without a selection agent;
- d) illuminating the treated and cultured plant material with an appropriate light source corresponding to the selection marker, wherein the selection marker is a visual marker; and
- e) selecting the plant material that shows the visual marker.

54. (Currently amended) The method as claimed in claim 41 ~~43~~, wherein ~~the~~ DNA targeting segments that allow double homologous recombination of the DNA of interest with ~~a~~ a plastid genome of interest have ~~a DNA sequence~~ sequences that ~~is~~ are homologous to a part of the plastid genome.

55. (Currently amended) A method ~~for the transformation of plastid genomes of a plant species, comprising the steps of:~~ as claimed in 43, wherein a set of DNA segments is selected from the group consisting of the *trnI* (*oriA*)/*trnA* region and the 16S/*trnV*/ORF70B region of a lettuce chloroplast genome.

- ~~a) providing a transformation vector carrying a DNA sequence of interest;~~
- ~~b) subjecting a plant material derived from *Asteraceae*, which comprises plastids, to a transformation treatment in order to allow the plastids to receive the transformation vector;~~
- ~~c) placing the thus treated plant material for a period of time into contact with a culture medium without a selection agent;~~
- ~~d) subsequently placing the plant material into contact with a culture medium comprising a selection agent; and~~
- ~~e) refreshing the culture medium comprising a selection agent to allow plant material comprising plastids that have acquired the DNA of interest to grow into transformants;~~

~~wherein the DNA segments that allow double homologous recombination of the DNA of interest with the a plastid genome of interest have a DNA sequence sequences that is or are homologous to a part of the plastid genome, and~~

~~wherein the set of DNA segment is selected from the group consisting of the *trnI* (*oriA*)/*trnA* region and the 16S/*trnV*/ORF70B region of a lettuce chloroplast genome.~~

56. (Currently amended) The method as claimed in claim 55 ~~41~~, wherein the a set of DNA segments is selected from the group consisting of SEQ ID NOs: 6, 7, 8, 9, 13, 14, 15 and 16. SEQ ID NO:6 and SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9, SEQ ID NO:13 and SEQ ID NO:14, SEQ ID NO:15 and SEQ ID NO:16.

57. (Previously presented) The method as claimed in claim 41, wherein the transformation treatment is selected from the group consisting of electroporation, particle gun transformation, polyethylene glycol transformation and whiskers technology.

58. (Previously presented) The method as claimed in claim 41, wherein the transformation treatment is polyethylene glycol transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 1 to 14 days.

59. (Previously presented) The method as claimed in claim 41, wherein the transformation treatment is polyethylene glycol transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 3 to 7 days.

60. (Previously presented) The method as claimed in claim 41, wherein the transformation treatment is polyethylenec glycol transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is about 6 days.

61. (Previously presented) The method as claimed in claim 41, wherein the transformation treatment is particle gun transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 1 to 14 days.

62. (Previously presented) The method as claimed in claim 41, wherein the transformation treatment is particle gun transformation and the period of time during which the

treated plant material is placed into contact with a culture medium without selection agent is 1 to 5 days.

63. (Previously presented) The method as claimed in claim 41, wherein the transformation treatment is particle gun transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is about 2 days.

64. (Previously presented) The method as claimed in claim 41, wherein the plant material to be treated is selected from the group consisting of plant tissue, separate cells, protoplasts and separate plastids.

65. (Cancelled)

66. (Previously presented) The method as claimed in claim 41, wherein step c) is performed in the dark.

67-81. (Cancelled)

82. (Previously presented) A transplastomic plant or plant part obtainable by the method as claimed in claim 41.

83. (Previously presented) A transplastomic plant or plant part as claimed in claim 82 wherein the plant or plant part is transformed by a vector comprising:

an expression cassette which comprises optionally a promoter active in the plastids of the plant species to be transformed, a DNA insertion site for receiving the transforming DNA of interest, optionally one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a DNA sequence encoding a transcription termination region active in the plastids of the plant species to be transformed, and

optionally a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with the plastid genome of interest

wherein the DNA segments that allow double homologous recombination of the DNA of interest with the plastid genome of interest have a DNA sequence that is homologous to a part of the plastid genome.

84. (Previously presented) The transplastomic plant or plant part as claimed in claim 82, wherein the plant is a lettuce plant.

85. (Cancelled)

86. (Currently amended) A progeny of a plant or plant part as claimed in claim 82, carrying plastids at least part of which have ~~the~~ a gene of interest in their genome.

87. (Previously presented) A progeny of a plant or plant part as claimed in claim 83, carrying plastids at least part of which have the gene of interest in their genome.

88. (Currently amended) ~~Plant parts~~ Plant part as claimed in claim 82, which plant ~~parts are~~ part is selected from the group consisting of tissues, cells, meristems, calli, protoplasts, plastids, proplastids and plastid DNA.

89. (Previously presented) Plant parts as claimed in claim 83, which plant parts are selected from the group consisting of tissues, cells, meristems, calli, protoplasts, plastids, proplastids and plastid DNA.

90. (Previously presented) The method as claimed in claim 48, wherein the therapeutic or prophylactic (bio)pharmaceutical (poly)peptide is an edible vaccine.

91. (Previously presented) The method as claimed in claim 52, wherein the visual marker is a fluorescent marker.

92. (Previously presented) The method as claimed in claim 91, wherein the fluorescent marker is green fluorescence protein.